ARCTIC MEDICINE

O.V. Zubatkina, L.K. Dobrodeeva, S.D. Kruglov

CHANGES IN IMMUNE PARAMETERS AND LYMPHOCYTE ATP LEVEL OF PERIPHERAL BLOOD IN RESIDENTS OF THE NORTH DURING SHORT-TERM COLD EPXPOSURE TO THE BODY

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The immune system constantly responds to various environmental stimuli. The aim of the study was to investigate immune effects of short-term cold exposure taking into account ATP level in peripheral blood lymphocytes in healthy residents of the North. So, the total number of lymphocytes and their phenotypes, as well as the content of cytokines and the concentration of ATP in the lymphocytes were determined in 38 volunteers twice (before and after their short-term stay for 5 minutes in a cold chamber at t = -25°C). Cluster analysis revealed two statistically different groups. The first group with an initially higher ATP level in lymphocytes responded to hypothermia by lower ATP concentration with the unchanging total number of lymphocytes as well as by a decrease in predominantly $CD16^+$ killer-cells. The other group reacted by an increase in ATP concentration with a decrease in the number of lymphocytes and by a pronounced decrease in $CD4^+$ helper-cells and in $CD71^+$ cells with a transferrin receptor. Also, the proinflammatory cytokines TNFα and IL-6 increased in the first group, while the second group showed a decrease in the level of lymphocyte-activating cytokine IL-1β. It can be assumed that the response to hypothermia in the first group is provided through the CIRP-NFkB-TNFα axis and leads to an increase in the risk of non-infectious inflammation. For the second group, a protective mechanism is triggered to restrain lymphocyte activity and the development of T-cell-mediated inflammation through regulation by means of T-effector and T-regulatory cells AMPK balance, autophagy, mitophagy and mitochondrial biogenesis. The study of the immune response to hypothermia is important for understanding the cellular mechanisms of adaptation as well as for the search of targets to correct the immune response.

Keywords: hypothermia, adaptation, T-cells, signaling mechanisms, ATP.

Introduction. A key mechanism of adaptive process is a rapid initiation of transient reactions responsible for homeostasis regulation, that is required to the body to adapt for continuously changing environment [10]. The immune system significantly impacts on the success of the body's adaptation to environmental factors and immune system responses to non-immunological stimuli continue to be comprehensively studied. Lymphocyte metabolism has sufficient plasticity to ensure demands of T cells for energy and biosynthesis, which considerably vary depending on their state and function. Intracellular metabolic regulators, in particular AMP-activated protein kinase (AMPK) being an energy sensor of the cell, play a key role in adaptation processes. AMPK is responsible for cell energy balance maintenance, by activating metabolic pathways of ATP production through oxidative phosphorylation (OXPHOX) and by inhibiting energy-consuming biosynthetic processes [7]. AMPK stimulates autophagia by means of positive regulation of ULK1 (unc-51-like autophagy-activating kinase 1) and formation of autophagosome complexes [2]. AMPK also facilitates mitophagy by triggering mitochondrial sorting through activation of MFF (mitochondrial fission factor) [11]. TRP-family receptors (transient receptor potential), being highly sensitive to environmental changes, play an important role in metabolic rearrangement of T cells [3]. TRP receptors (in particular TRPV3,4 and TRPM8) in addition to their main ion-channel function facilitate activation of cold-inducible proteins (CIPs), that protect cells during cold, hypoxia and other types of stress [8]. The study of immune responses to cold exposure is of prime importance for better understanding of adaptation mechanisms to the North environment and for developing ways to increase the body's resistance to low temperatures.

The aim of the study was to investigate short-term cold exposure effect on immune system taking into consideration ATP level in peripheral blood lymphocytes in healthy residents of the North.

Materials and methods 38 volunteers, residents of the Arkhangelsk city of both sexes, were examined. The average age was 35 (8.7) years. All volunteers gave their consent to participate and were informed about the results of the study in accordance with the requirements of the Declaration of Helsinki (the World Medical Association) on the ethical principles of medical research (2000).

Venous blood was collected twice, before and after the volunteers underwent short-term cold exposure in a cold chamber (time duration – 5 min; temperature – minus 25 ˚C). In both samples, total number of lymphocytes and their individual phenotypes were evaluated. The content of cytokines and adenosine triphosphate (ATP) in lymphocytes were also measured.

The complex of immunological studies included isolation of lymphocytic fraction from the peripheral blood followed by identification of lymphocyte phenotypes ($CD3^+$, $CD4^+$, $CD8^+$, $CD10^+$, $CD16^+$, $CD71^+$, $CD23^+$, $CD25^+$, $CD95^+$, HLA DR) by indirect immuno-peroxidase reaction (reagents of Sorbent LLC, Russia). The concentration of cytokines (IL-1β, IL-6, TNF-α, IL-10) in blood serum was measured by ELISA assay on an automatic analyzer "Evolis" "Bio-RAD" (Germany). ATP concentration in lymphocytes was

ZUBATKINA Olga Vladimirovna - PhD, professor, research scientist of the laboratory Ecological immunology Institute of Environmental Physiology of N. Laverov Federal Center for Integrated Arctic Research Ural Department of Russian Academy of Sciences. Address: 163061, Arkhangelsk, Lomonosov ave., 249. Phone number: +79214953695, e-mail: ozbiochem@gmail.com. SPIN 1581-5178; ORCID 0000-0002-**5039-2220, DOBRODEEVA Liliya Konstantinovna** - professor, chief scientist of the laboratory Ecological immunology Institute of Environmental Physiology of N. Laverov Federal Center for Integrated Arctic Research Ural Department of Russian Academy of Sciences. Address: 163061, Arkhangelsk, Lomonosov ave., 249. Phone number: 65-29-95, e-mail: dobrodeevalk@mail.ru. SPIN 4518-6925; ORCID 0000-0001-5080-6502, **KRUGLOV Sergey Dmitrievich** - full-time postgraduate student of the laboratory Ecological immunology Institute of Environmental Physiology of N. Laverov Federal Center for Integrated Arctic Research Ural Department of Russian Academy of Sciences. Address: 163061, Arkhangelsk, Lomonosov ave., 249. Phone number: +79210782348, e-mail: stees67@yandex.ru. SPIN 2532-9912; ORCID 0000-0002-4085-409X

measured on luminometer by bioluminescence using luciferin-luciferase reaction (reagents Lumtek LLC (Russia)).

Statistical analysis was performed using «Statistica 10.0» software («StatSoft», USA). In the multivariate exploratory analysis module, the data was clustered using the "K means" method. In the descriptive statistics module, mean values (M) and standard deviation (SD) were calculated; the Kolmogorov-Smirnov and Lilliefors test for normality was used to check the data for normality of distribution. When the distribution was close to normal, the Student's t-test was used to compare the results of the samples, the differences were considered significant at p<0,05.

Results. A statistical analysis of the obtained data using descriptive statistics and clustering by the "K-means" method helped us to reveal two groups. These groups were statistically different in lymphocyte ATP level and in the most of the determined parameters, which changed ambiguously after cold exposure (table 1).

The data in the table shows that after a cold exposure in the first cluster group, the ATP level in lymphocytes significantly decreased, while their total number in peripheral blood did not change. Concentration of helper-cells ($CD4^+$), killer-cells ($CD16^+$) and cells with IL-2 receptor ($CD25^+$) significantly reduced, however, the content of the other determined phenotypes remained at the same level. In the second cluster group concentration of ATP after a cold exposure, on the contrary, increased against significant decrease in both the total number of lymphocytes and all determined phenotypes. Changes in the total number of lymphocytes and the level of ATP in lymphocytes during cold exposure is shown in the diagram (Fig. 1)

In the plasma cytokine profile of the first group, the background concentration of the pro-inflammatory cytokines IL-6 and TNF-α significantly increased relative to their values before cold exposure. In the second group, the concentration of the lymphocyte-activating factor IL-1β decreased, and the level of the other cytokines did not change.

The calculation of relative content of lymphocyte phenotypes within the groups showed that the proportion of the majority of phenotypes in the groups was equal. However, in the first cluster group, compared to the second cluster group, the content of $CD16^+$ and $CD25^+$ cells were higher on 4.6 and 2.4% respectively and the content of $CD71^+$ cells with a transferrin receptor was lower on 2.5%. After cold exposure some changes were observed in the content of lymphocytes, which had their own characteristics for each group. In the first group, the content of $CD16^+$ killer-cells, $CD4^+$ helper-cells, and activated $CD25^+$ cells with IL-2 receptor predominantly decreased by 5.8, 3.9, and 3%, respectively (Fig. 2). In the second group, the proportion of $CD4^+$ cells decreased significantly (by 11.2%), the content of $CD8^+$ cytotoxic cells and $CD71^+$ cells with a transferrin receptor decreased by 4.4 and 4.7%, respectively (Fig. 2). For the other types of cells, the differences were smaller both within each group and between groups.

Discussion of results. An adaptive reaction to cold exposure leads to the induction of the so-called cold shock proteins. As soon as the body is exposed to low temperatures, these specific proteins immediately react to allow cells to quickly adapt to environmental conditions. One of them is the cold-inducible RNA binding protein (CIRP) which activity is enhanced by hypothermia and, in addition, it promotes translation of some specific mRNAs [17]. It is estimated that at low temperatures, regardless of the global suppression of protein expression, transcription of CIRP mRNA is significantly increased due to the CIRP-mediated transcriptional activation of alternative promoters [1]. Enhanced expression of CIRP leads to its accumulation in the cytoplasm, where CIRP exerts its cytoprotective effect: it increases the activation of anti-apoptotic proteins Bcl-2 and Bcl-x1 through activation of the MAPK / ERK1/2 pathway and nuclear factor NF-kB [5] and inhibits caspase cascade through suppression of Bax and Bad pro-apoptotic factors [9]. In addition, CIRP promotes

Changes of measured parameters in groups before and after cold exposure

Parameters	before cold exposure			after cold exposure			
	cluster 1 (N=11)	cluster 2 (N=27)	Signif. level P	cluster 1 (N=11)	Signif. level P*	cluster 2 (N=27)	Signif. level P**
ATP μmol /106 cells	3.81 (1.389)	0.77 (0.563)	0.00002	2.59 (1.415)	0.0272	1.58 (1.544)	0.0071
Lymph×106 cells/ml	1.06 (0.344)	1.81 (0.780)	0.0002	0.94 (0.260)	0.187	1.34 (0.503)	0.0087
CD4 ×106 cells/ml	0.21 (0.085)	0.34 (0.176)	0.0052	0.15 (0.080)	0.0436	0.10 (0.087)	0.0002
CD8 ×106 cells/ml	0.20 (0.102)	0.31 (0.149)	0.0095	0.15 (0.077)	0.119	0.17 (0.073)	0.0001
CD10 ×106 cells/ml	0.20 (0.067)	0.32 (0.123)	0.0008	0.15 (0.089)	0.094	0.20 (0.093)	0.0004
CD95 ×106 cells/ml	0.15 (0.058)	0.27 (0.103)	0.0007	0.12 (0.051)	0.142	0.17 (0.083)	0.0011
CD16 ×106 cells/ml	0.23 (0.105)	0.31 (0.144)	0.058	0.15 (0.065)	0.0257	0.19 (0.093)	0.0008
CD23 ×106 cells/ml	0.18 (0.058)	0.32 (0.131)	0.0004	0.17 (0.113)	0.372	0.22 (0.107)	0.0069
CD25 ×106 cells/ ml	0.20 (0.061)	0.30 (0.142)	0.0072	0.15 (0.061)	0.0415	0.21 (0.104)	0.0143
CD71 ×106 cells/ml	0.19 (0.058)	0.37 (0.163)	0.00002	0.15 (0.072)	0.083	0.21 (0.091)	0.00003
HLAD×106 cells/ml	0.21 (0.081)	0.32 (0.153)	0.0073	0.18 (0.093)	0.267	0.23 (0.114)	0.0205
IL-1β pcg/ml	5.34 (0.497)	5.88 (0.876)	0.0250	5.36 (0.303)	0.454	5.40 (0.713)	0.0163
IL-6 pcg/ml	0.57 (0.416)	1.18 (0.877)	0.0081	1.41 (0.563)	0.0005	1.28 (1.141)	0.365
IL-10 pcg/ml	3.04 (1.549)	4.20 (3.159)	0.139	2.90 (2.128)	0.434	3.57 (2.852)	0.225
TNF-α pcg/ml	1.20 (0.867)	2.11 (1.544)	0.0398	2.18 (1.209)	0.0287	2.49 (2.535)	0.279

- significance level relative to values before cold exposure of *- cluster 1,**- cluster 2.

the activation of antioxidant defense, reducing the negative effects of reactive oxygen forms, production of which increases under conditions of cold stress [16]. At the same time, getting into the circulation by lysosomal secretion, CIRP manifests itself as a Danger-associated molecular pattern (DAMP) in a non-cellular environment. By interacting with TLR4 receptors and leading, through stimulation of NF-kB, to the production of pro-inflammatory cytokines CIRP is capable of initiating a non-infectious inflammatory response [4,16]. Obtained results showed an increase in the content of pro-inflammatory cytokines (TNF-α and IL-6) in the blood of the first group of volunteers.

Lymphocytes need a certain balance of production and energy consumption associated with their current state, differentiation and functioning. The fluctuations of the relative content of lymphocyte phenotypes in groups with different background levels of ATP and its change in response to cold are noteworthy. In the first cluster group, where initial ATP level of lymphocytes was high, the significant decrease was in content of $CD16^+$ killer-cells with a decrease in the concentration of ATP in lymphocytes. While in the second cluster group (with an initially low ATP level) there was a sharp decrease in the content of $CD4^+$ helper-cells with an increase in lymphocytes ATP concentration. AMPK plays an important role in T-cell metabolism, affects T-cell development and fate, and has both positive and negative effects in relation to growth, differentiation and functions of T-cells [15]. Suppressing the activity of mTOR complex 1 and acetyl-coenzyme A carboxylase, AMPK inhibits energy-consuming syntheses of proteins and fatty acids, reducing the growth and functioning of T-effector cells [14]. On the other hand, by stimulating β-oxidation of fatty acids and OXPHOS in $CD4^+$ cells, AMPK directs cell differentiation from Th17 to Treg cells [6], influences T-cell-mediated inflammation through a change in the balance of T-effector and T-regulatory cells [13]. AMRK also stimulates autophagy (through activation of ULK1) [2], mitophagy (through the induction of mitochondrial factor MFF) [11], mitochondrial biogenesis (via direct and NAD^+-mediated SIRT1 regulation of the mitochondrial modulator PGC-1α) [4], and thus, contributes to the increase in ATP production in cells. An increase in ATP level in lymphocytes was observed in the second group.

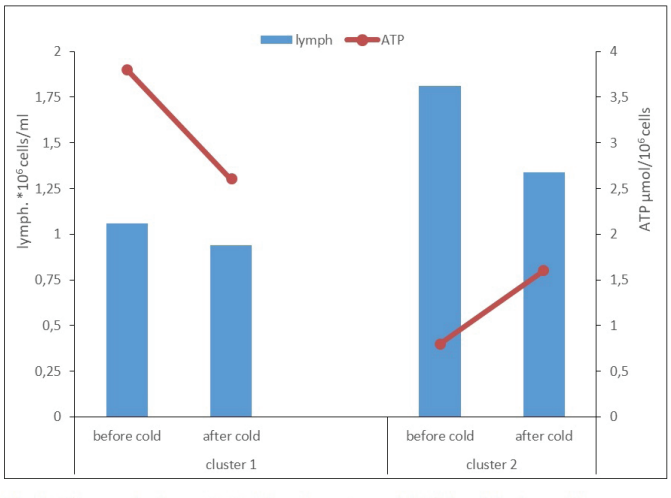

Fig. 1. Changes in the content of lymphocytes and ATP level during cold exposure.

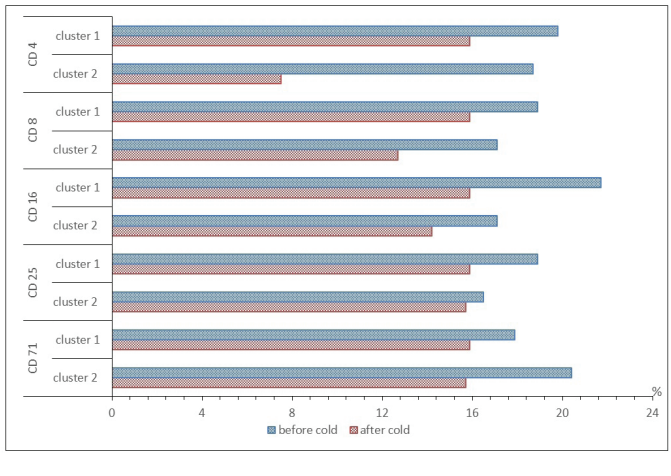

Fig. 2. Changes in content of individual lymphocyte phenotypes in cluster groups.

Conclusion. The immune response on influence of low temperature is expressed in quantitative changes of T cells, their phenotypes and cytokines and is associated with intracellular ATP level. The results show that after cold exposure in the group with an initially higher level of ATP, the levels of pro-inflammatory cytokines (IL-6 and TNF-α) increase. Also, the content of mainly $CD16^+$ killer-cells decreases and the concentration of ATP in lymphocytes decreases while the total number of lymphocytes in the peripheral blood does not change. In the group with lower ATP values, the level of lymphocyte-activating IL-1β decreases, the content of $CD4^+$ helper-cells and activated $CD71^+$ cells with a transferrin receptor decreases significantly. At the same time, the concentration of ATP in lymphocytes increases with a decrease in the total number of lymphocytes in the blood. These differences may be due to the response associated with the energy status of cells, metabolic pathway activity, and transduction signals. It can be assumed that for the first group, the action of cold leads to the production of pro-inflammatory cytokines through the axis of TRP - CIRP - NF-kB - TNF-α and a decrease in the lymphocytes ATP level. For the second group, it is possible that a mechanism of restraining lymphocytes activity and T-cell-mediated inflammation is triggered through AMPK regulation presenting an increase in ATP concentration accompanied by the decrease in the total number of lymphocytes and their phenotypes.

A more detailed study of the T-cell response to low temperature exposure can provide a better understanding of adaptation mechanisms and detection of targets for specific correction of possible immune disorders in people living in the North.

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V.P. Novitskaya, E.I. Prakhin

AGE-RELATED FEATURES OF THE ACTIVITY OF LYMPHOCYTE ENZYMES AND THEIR INTERCONNECTION IN CHILDREN OF THE FAR NORTH

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The research was conducted to study activity indicators of lymphocyte enzymes in 99 healthy children aged 3 to 15 years living in town Tynda in the summer season. The increase of enzymes activity in children's lymphocytes was noted as they mature. Differences in indicators of enzyme activity were manifested in a lower level of dehydrogenases in the group of 3-year-old children, higher acid phosphatase, and lower glycerol-3-phosphate dehydrogenase in all age groups. Some features of age-related dynamics of the correlation relationships of the studied indicators are noted and periods of greatest adaptive tension in children are determined.

Keywords: North, children, lymphocytes, enzymes, correlation

Introduction. The regions of the Far North and equivalent areas cover more than 64% of the territory of Russia [11]. Issues of human full life activity and protection of his health in extreme climatic conditions of the North are far from being resolved. The health of the population, especially children's is under the constant influence of changeable parameters of the climate system, which often leads to the formation of various pathologies.

The city Tynda, in which the studies were conducted, is located in the most northern city of the Amur Region and, according to a number of climatic and geographical features, is assigned to areas equated to the North.

For assessing a degree of impact of the extreme environment per person, the characteristics of homeostatic systems reflecting the adaptive capabilities of the organism should be included. Blood cells are considered as components of the immune system involved in adaptive reactions [4].

Lymphocytes are the main morphological substrate of the immune system. And the regulation of the immune response is determined by functional capabilities of these cells, which are based on intracellular metabolic reactions. The latter ones, to a large extent, are provided by a certain level of intracellular enzymes activity [2,16].

The level of functioning of these cells is supported by the mechanisms of neuroendocrine regulation, in the complex interaction of intracellular relationships, wherein the variability of the level of these connections is the most important reserve of target results effects on the organism [3].

The urgency of the problem lies in the fact that children's organism is labile to the effects of the environment and its climatic features. Children of preschool and school age are characterized, on the one hand, by intensive growth and development rates, and, on the other hand, by insufficiently high resistance to adverse factors during this period of ontogenesis.

We have chosen the metabolic parameters of blood lymphocytes and the correlation between them as integral indicators of the degree of environmental impact on the organism. A change in the correlations between the physiological parameters of the organism under the influence of various systems of adverse environmental factors has been repeatedly proven in adult populations [3, 5, 10].

Studies dedicated to the study on the characteristics of the activity of enzymes of lymphocytes, taking into account their relationships in children in the North, are few and poor in content.

Aim of the study: to reveal the features of age-related dynamics of the activity of lymphocyte enzymes and their relationships in children of the alien population of the North.

Material and methods of research. 99 children aged from 3 to 15 were examined years living in the city of Tynda, Amur Region. All children were divided into groups: 3, 5, 7, 10 and 15 years. The

NOVITSKAYA Valentina Petrovna – Doctor of Biological Sciences, Senior Researcher Clinical Pathophysiology Laboratories FRC KSC SB RAS Research Institute of Medical Problems of the North, Krasnoyarsk, E-mail:impn@impn.ru, ORCID: 0000-0002-7556-7699, **PRAKHIN Efim Isaakovich** – Doctor of Medical Sciences, Professor, Chief Researcher, E-mail:eprakhin@yandex.ru, ORCID: 0000-0003-1583-9928